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Investigating the microbiome of the bovine uterus in relation to endometritis, a costly disease for dairy farmers

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Endometritis is inflammation of the inner lining of the uterus, affecting up to 20% of the dairy cows after calving in Denmark. The disease causes reduced pregnancy rates, which often leads to culling of the cows and is costly for the farmer. Until now, investigations of which pathogens may cause the disease have been based on microbiological culturing, and no conclusive evidence has been found. Only a fraction of the bacterial flora is cultivable, and therefore more than 90% of the uterine microbiome has not been characterised. With incomplete knowledge of the pathogens, treatment is performed without an option for choosing the best suited antimicrobial agent, which may lead to unnecessary antibiotic resistance development. The present study is based on 16S rRNA PCR, which in combination with 454 next generation sequencing allows phylogenetic identification of the bacteria present in the sample. Not being limited to bacteria that are suited to growth under laboratory conditions, this study promises a more comprehensive insight into the microbiome of the dairy cow uterus than has previously been offered.

Cows (n=40) on a Danish dairy herd were randomly selected on the basis of a uterine score indicating that the cows had uterine pathology. Uterine fluid was aspirated and if necessary the uterus was flushed with 30 ml sterile saline solution in order to retrieve uterine material. The fluid was placed in RNeasy lysis buffer. An endometrial biopsy was retrieved and the tissue placed in RNeasy lysis buffer. The cows were sampled on days 5-11 (week 1), days 26-32 (week 4), and on days 47-53 (week 7). This sampling schedule provided an opportunity to follow the development of any infection, and the combination of biopsy and uterine flush samples offered insights into whether tissue-invasive bacteria were present. The DNA was extracted with the Maxwell 16 LEV Blood kit (Promega), the 16S rRNA PCR was performed with primers targeting the V2 region, and the 454 next generation sequencing was performed by GATC.

Previous papers based on culturing of endometrial swabs or biopsies point to *Escherichia coli*, *Trueperella* (*Archanobacterium*) *pyogenes*, and *Fusobacterium necrophorum* as the most likely pathogens, although some of them also seem to be present in healthy animals. We expect to find these bacteria in the samples from the diseased animals, and perhaps the detailed data from the sequencing will also reveal hitherto undiscovered pathogens.